

What is claimed is:

1. A biologically-active conjugate comprising a biologically-active molecule having a reactive thiol moiety and a non-peptidic polymer having an active sulfone moiety forming a linkage with said thiol moiety.

*thiol - polymer*

2. The biologically-active conjugate of claim 1, wherein said active sulfone moiety is vinyl sulfone.

3. The biologically-active conjugate of claim 1, wherein said active sulfone moiety is chloroethyl sulfone.

4. The biologically-active conjugate of claim 1, wherein said biologically-active molecule is a tumor necrosis factor (TNF) inhibitor.

5. The biologically-active conjugate of claim 4, wherein said TNF inhibitor is selected from the group consisting of a 30kDa TNF inhibitor, a 40kDa TNF inhibitor, a  $\Delta 51$  TNF inhibitor, and a  $\Delta 53$  TNF inhibitor.

6. The biologically-active conjugate of claim 5, wherein said TNF inhibitor is the 30kDa TNF inhibitor.

7. The biologically-active conjugate of claim 5, wherein said TNF inhibitor is the 40kDa TNF inhibitor.

8. The biologically-active conjugate of claim 5, wherein said TNF inhibitor is the  $\Delta 51$  TNF inhibitor.

9. The biologically-active conjugate of claim 5, wherein said TNF inhibitor is the  $\Delta 53$  TNF inhibitor.

5 10. The biologically-active conjugate of claim 1, wherein said biologically-active molecule is an interleukin-1 (IL-1) inhibitor.

11. The biologically-active conjugate of claim 10, wherein said IL-1 inhibitor is interleukin-1 receptor antagonist (IL-1ra).

12. A method of preparing a biologically-active conjugate, comprising the steps of:

- 10 (a) reacting a biologically-active molecule having a reactive thiol moiety with a non-peptidic polymer having an active sulfone moiety to form said conjugate; and  
(b) optionally, isolating said conjugate.

13. The method of claim 12, further comprising, before step (a), the steps:

- 15 selecting a desired biologically-active molecule; and  
adding a reactive thiol moiety to the selected molecule to form a biologically-active molecule having a reactive thiol moiety.

14. The method of claim 12, further comprising, before step (a), the steps of:

selecting a biologically-active molecule;

adding a reactive thiol moiety to the selected molecule to form a synthetic molecule;

and

5 refolding the synthetic molecule to form a biologically-active molecule having a reactive thiol moiety; and

optionally, isolating the biologically-active molecule having a reactive thiol moiety.

15. A substantially purified compound of the formula  $R_1-X-R_2$ , wherein:

X comprises a non-peptidic polymer having a first reactive group and a second reactive group, wherein said first reactive group is a Michael acceptor;

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 $R_1$  comprises a biologically-active molecule having a reactive thiol moiety, said biologically-active molecule is covalently bonded to said non-peptidic polymer by reaction of said thiol moiety with said Michael acceptor, and said biologically-active molecule retains its biological activity after said reaction, and

15  $R_2$  comprises a biologically-active molecule or a nonbiologically-active group bonded to said non-peptidic polymer by reaction with said second reactive group.

16. The substantially purified compound of claim 15, wherein said Michael acceptor is vinyl sulfone.

17. The substantially purified compound of claim 15, wherein said Michael acceptor is maleimide.

18. The substantially purified compound of claim 15, wherein said non-peptidic polymer has two Michael acceptors.

19. The substantially purified compound of claim 18, wherein said Michael acceptors are maleimide.

5 20. The substantially purified compound of claim 18, wherein said Michael acceptors are vinyl sulfone.

21. The substantially purified compound of claim 18, wherein one of said Michael acceptors is vinyl sulfone and the other is maleimide.

10 22. The substantially purified compound of claim 15, wherein said biologically-active molecule is selected from the group consisting of an IL-1 inhibitor, a tumor necrosis factor binding protein (TNFbp), CR1, PDGF receptor, IL-2, and exon 6 peptide of PDGF.

23. The substantially purified compound of claim 22, wherein said biologically-active molecule is a tumor necrosis factor binding protein (TNFbp).

15 24. The substantially purified compound of claim 23, wherein said TNFbp is the 30kDa TNF inhibitor.

25. The substantially purified compound of claim 23, wherein said TNFbp is the 40kDa TNF inhibitor.

26. The substantially purified compound of claim 23, wherein said TNFbp is the  $\Delta 51$  TNF inhibitor.

27. The substantially purified compound of claim 23, wherein said TNFbp is the  $\Delta 53$  TNF inhibitor.

28. The substantially purified compound of claim 22, wherein said biologically-active molecule is an IL-1 inhibitor.

29. The substantially purified compound of claim 28, wherein said IL-1 inhibitor is IL-1ra.

30. The substantially purified compound of claim 15, wherein  $R_1$  comprises a biologically-active polypeptide.

31. The substantially purified compound of claim 15, wherein  $R_1$  and  $R_2$  comprise biologically-active polypeptides.

32. The substantially purified compound of claim 15, wherein  $R_1$  and  $R_2$  are identical.

33. The substantially purified compound of claim 15, wherein  $R_1$  and  $R_2$  are different.

34. A water-soluble polymer having a reactive NHS-ester and a reactive Michael acceptor.

35. The water-soluble polymer of claim 34, wherein said Michael acceptor is vinyl sulfone.

36. The water-soluble polymer of claim 34, wherein said Michael acceptor is maleimide.

37. The water-soluble polymer of claim 34, wherein said polymer is selected from the group consisting of polyalkylene oxides, polyoxyethylated polyols, and polyolefinic alcohols.

5 38. A method for the preparation of the substantially purified compound of claim 15, comprising:

(a) reacting X with  $R_1$  and  $R_2$  to form  $R_1$ -X- $R_2$ ; and

(b) purifying  $R_1$ -X- $R_2$ .

10 39. The method of claim 38, further comprising adding a reactive thiol moiety to a biologically-active molecule to form  $R_1$  prior to step (a).

40. The method of claim 38, further comprising, prior to step (a), the steps:

selecting a biologically-active molecule;

adding a reactive thiol moiety to the selected molecule to form a synthetic molecule;

15 refolding the synthetic molecule to form  $R_1$ ; and

optionally, isolating  $R_1$ .

41. The method of claim 38, wherein step (a) further comprises the steps:

protecting a reactive group of X to form a protected group on X;

reacting X having a protected group with  $R_1$  to form  $R_1$ -X;

20 deprotecting the protected group on X;

reacting  $R_1$ -X with  $R_2$  to form  $R_1$ -X- $R_2$ .

42. The method of claim 38, wherein step (a) further comprises the steps:

reacting an excess of X with  $R_1$  to form  $R_1-X$ ; and

reacting  $R_1-X$  with  $R_2$  to form  $R_1-X-R_2$ .

43. A pharmaceutical composition comprising the compound of claim 1 in a  
5 pharmaceutically-acceptable carrier.

44. A pharmaceutical composition comprising the compound of claim 15 in a  
pharmaceutically-acceptable carrier.

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